1,172,800

# PATENT SPECIFICATION

NO DRAWINGS

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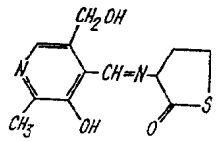
COMPLETE SPECIFICATION

## Novel N-Pyridylmethylidene-Homocysteine Thiolactone Compound and the preparation thereof

We, TANABE SEIYAKU COMPANY LIMITED, a Company registered under the Laws of Japan, of 21 Dosho-machi 3-chome, Higashi-ku, Osaka, Japan, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to a novel N-pyridylmethylidene-homocysteine thiolactone compound and to the process for preparing the same. More particularly, it relates to N-(2-methyl-3-hydroxy-5-hydroxymethyl-4-pyridylmethylidene)-homocysteine thiolactone.

The compound may be illustrated by the following formula.



(I)

It has been well known that vitamin $B_6$ compounds for example pyridoxine, pyridoxal and pyridoxamine, which are important for therapy and nutrition, have the disadvantageous property of a low absorption and short duration in the living body. Therefore, it has been desired to obtain vitamin $B_6$ derivatives having higher absorbability and longer durability in the living body.

We have now found that the compound (I) of this invention has not only the activity of both vitamin $B_6$ and homocysteine but also a very high and durable pyridoxal level in the living body as compared with those known vitamin $B_6$ compounds.

When administering the compound (I) of this invention orally, it is readily absorbed and results in a higher and more durable pyridoxal level in the living body than pyridoxal hydrochloride. This fact is clearly proved by the following experimental data shown in Table I, where vitamin $B_6$ derivatives tabulated in Table I were administered orally to male white rabbits of 2.0—2.5 kg. body weight at equimolar doses to pyridoxal hydrochloride, and the subsequent vitamin $B_6$ level (γ/ml.) in the blood was measured for a period of time by the microbiological method (using Saccharomyces carlsbergensis).

TABLE I

| Vitamin $B_6$ derivatives | Time after oral administration | | | |
|---|---|---|---|---|
| | 30 min. | 1 hr. | 2 hrs. | 3.5 hrs. |
| The compound (I) of this invention in the form of free base | 210 | 300 | 280 | 290 |
| The hydrochloride of the compound (I) of this invention | 60 | 350 | 290 | 385 |
| Pyridoxal hydrochloride | 310 | 256 | 128 | 90 |

On the other hand, $LD_{50}$ of the compound (I) of this invention are illustrated in Table II.

TABLE II

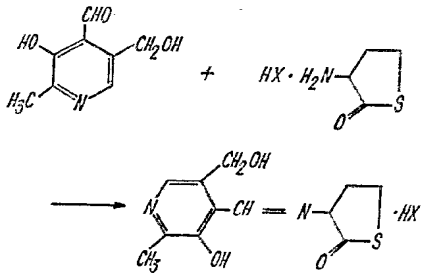
| Vitamin $B_6$ derivatives | $LD_{50}$ (mg./kg.) oral administration | $LD_{50}$ (mg./kg.) subcutaneous injection |
|---|---|---|
| The compound (I) of this invention in the form of free base | >3000 | >3000 |
| The hydrochloride of the compound (I) of this invention | >3000 | 1900 |
| Pyridoxal phosphate | >3000 | 550 |

There is observed acute toxic symptoms such as hyperventilation, clonus and sensitivity of sounds in the subcutaneous administration of pyridoxine hydrochloride or pyridoxal phosphate , but the aforementioned symptoms were not pronounced in the oral and subcutaneous administration of the compound (I).

From the foregoing fact, the compound (I) of this invention is more useful than vitamin $B_6$ compounds which are known heretofore for the treatment of vitamin $B_6$ deficiency symptoms, particularly on its oral administration.

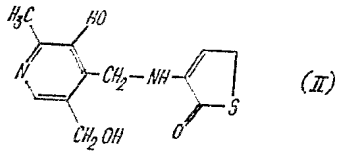
The compound of this invention is also useful, due to the synergetic activity of vitamin $B_6$ and homocysteine, for prevention and treatment of various dermatitis such as seborrhoetic dermatitis, acne simplex or INAH (isonicotinic acid hydrazide) eruption.

According to the present invention, the compound (I) can be prepared by the method represented as follows:



wherein HX is an acid. The condensation reaction may be conveniently carried out by mixing pyridoxal and an acid addition salt of homocysteine thiolactone in a suitable solvent, and stirring the mixture for a few hours, preferably at room temperature. Methanol, ethanol or isopropanol may be used as the reaction solvent. Pyridoxal in the above reaction can be replaced with an acid addition salt of pyridoxal or (3-hydroxy-5-hydroxymethyl - 2 - methyl - 4 - pyridyl) - hydroxymethane sulfonic acid in the betaine form or its alkali metal salt, e.g. the sodium salt. The oxidation mixture of pyridoxine or the reaction mixture of pyridoxal oxime with nitrous acid, in which pyridoxal is being formed, can also be employed as the starting material. The final product (I) can be recovered from the reaction mixture conveniently in the form of an acid addition salt e.g. the hydrochloride or sulfate. Alternatively, the final product (I) can be easily recovered in the form of a free base by adding metal alkoxide, e.g. sodium methoxide to the reaction mixture. The precipitating crystals of the final product (I) may be collected and purified in conventional manners.

We have assumed that the compound of this invention has the structure of the following tautomeric form (II), because the signal ($\tau$, in DMSO $d_6$: 3.90, 1H (Triplet, $J=3$ c/s)) of the proton of olefine was observed in the spectrum of the nuclear magnetic resonance of said compound instead of the proton of azomethine, and the spectrum of the ultraviolet absorption of said compound has a strong resemblance to that of pyridoxamine.



(II)

EXAMPLE 1.

2.0 g. of pyridoxal was suspended in 60 ml. of methanol. To the solution was added gradually 1.9 g. of homocysteine thiolactone hydrochloride while stirring at room temperature. The solution was stirred for an additional one and a half hours. The solution was

then allowed to stand in a refrigerator. The precipitating crystals were collected by filtration and dried, whereby yellow prisms of N-(2 - methyl - 3 - hydroxy - 5 - hydroxymethyl-4 - pyridylmethylidene) - homocysteine thiolactone hydrochloride were obtained.

Yield: 2.2 g. (61%) M.P. 180—182°C (decomp.)

Analysis calculated for $C_{12}H_{14}O_3N_2S$ HCl
C, 47.61 : H, 4.99 : N, 9.26
Found: C, 47.42 : H, 4.99 : N, 9.08

EXAMPLE 2.

10.0 g. of pyridoxal was suspended in 200 ml. of methanol. To the solution was added gradually 9.2 g. of homocysteine thiolactone hydrochloride while stirring. The solution was stirred for an additional one and a half hours. Then, sodium methoxide prepared by 1.38 g. of sodium and 50 ml. of methanol was added to the solution. While crystals were precipitating, the mixture was allowed to stand in a refrigerator for a night. Precipitated crystals were collected by filtration, washed with 200 ml. of cold water and a small amount of acetone successively. The crystals were recrystallized from methanol, whereby yellow prisms of N - (2 - methyl - 3 - hydroxy - 5-hydroxymethyl - 4 - pyridylmethylidene)-homocysteine thiolactone were obtained.

Yield: 8.5 g. (50%) M.P. 172—175°C (decomp.)

Analysis calculated for $C_{12}H_{14}O_3N_2S$
C, 54.13 : H, 5.30 : N, 10.52 : S, 12.04
Found: C, 54.32 : H, 5.40 : N, 10.28 : S, 11.98

The invention includes within its scope vitamin compositions which comprise a new active material according to the invention in association with a pharmacologically acceptable carrier. Such carrier may be, for example, an inert solid diluent, e.g. talc, provided to increase the bulk and so render the active material more easy to dispense, solid materials by which the active material is formed into pills or tablets or a liquid medium in which the active material is dissolved or suspended to render it suitable for administration by injection. The amount of active material present in the composition when it is provided in the form of dosage units such as pills, tablets or capsules should be arranged such that a patient's requirements can be met by a small number, preferably not more than six, dosage units per day. In the case of a composition for injection, the concentration should be such that the patient's requirements may be met conveniently by the injection of a whole number of half cubic centimetres per day.

WHAT WE CLAIM IS:—

1. N - (2 - methyl - 3 - hydroxy - 5-hydroxymethyl - 4 - pyridylmethylidene)-homocysteine thiolactone or an acid addition salt thereof.

2. N - (2 - methyl - 3 - hydroxy - 5-hydroxymethyl - 4 - pyridylmethylidene)-homocysteine thiolactone hydrochloride.

3. A process for preparing a compound according to claim 1, which comprises reacting pyridoxal in the form of the free base or an acid addition salt with an acid addition salt of homocysteine thiolactone, and if required neutralizing the product with a metal alkoxide.

4. A process for preparing a compound according to claim 1, which comprises reacting the betaine form of (2 - methyl - 3-hydroxy - 5 - hydroxymethyl - 4 - pyridyl)-hydroxy-methane sulfonic acid or an alkali metal salt thereof with an acid addition salt of homocysteine thiolactone, and if required neutralizing the product with a metal alkoxide.

5. A process according to claim 4 in which the acid addition salt of homocysteine thiolactone is the hydrochloride thereof.

6. A process for preparing a compound according to either of claims 1 or 2, substantially as hereinbefore described and illustrated by the foregoing Examples.

7. A compound according to claim 1 when prepared by a process in accordance with any one of claims 3 to 6.

8. A composition for the treatment of vitamin $B_6$ deficiency symptoms which comprises a compound acording to any one of claims 1, 2 or 7 in association with a therapeutically acceptable carrier.

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